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(57) Abstract

The invention relates to certain heteroaroyl-10-deazaaminopterin compounds, as well as a method and composition employing certain heteroaroyl-10-deazaaminopterin compounds for the treatment of inflammatory disease, such as rheumatoid arthritis.

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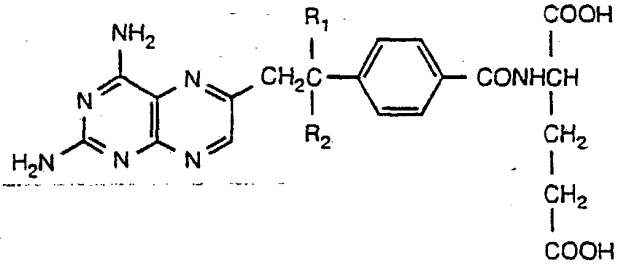
HETEROAROYL-10-DEAZAAMINOPTERINS FOR TREATMENT OF INFLAMMATION

Field of the Invention

The invention relates to certain heteroaroyl-10-deazaaminopterin compounds, as well as a method and composition employing certain heteroaroyl-10-deazaaminopterin compounds for the treatment of inflammatory disease, such as rheumatoid arthritis.

Background of the Invention

DeGraw et al., U.S. Patent No. 4,369,319, issued January 19, 1983, disclose 10-deazaaminopterin compounds having the structure:



In the compound 10-deazaaminopterin, R_1 and R_2 are both hydrogen. In the alkyl derivatives of 10-deazaaminopterin disclosed in Patent No. 4,369,319, either or both of R_1 and R_2 is alkyl having from one to about eight, preferably one or two carbon atoms. When only one of R_1 and R_2 is alkyl, the other is hydrogen. Exemplary R_1 and R_2 alkyl include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, amyl, iso-amyl, sec-amyl, tert-amyl, hexyl, iso-hexyl, heptyl, iso-heptyl, octyl, iso-octyl, 2-ethyl hexyl and tert-octyl.

DeGraw et al., J. Med. Chem., 17, 552 (1974), reported on the synthesis and antifolate activity of 10-deazaaminopterin. The antimicrobial and antitumor activities of the powerful dihydrofolic reductase inhibitors aminopterin and its N-10 methyl derivative, methotrexate, are well known, and numerous analogues have been made to further improve the potency, cell penetration and toxicity properties of these compounds. As part of a continuing program to investigate structure-activity relationships in folic acid analogues, DeGraw et al. were interested in the effects of replacement of the nitrogen atom in the side chain of aminopterin and reported on the synthesis and biological activity of 10-deazaaminopterin. Continuing work with 10-deazaaminopterin and its 10-alkyl derivatives led to the discovery of their antileukemic activity and to their efficacy in treating various ascites tumor systems.

In accordance with U. S. Patent 4,369,319, it was determined that leukemia, as well as other malignancies, including ascitic tumors, can be ameliorated in warm-blooded lower animals

by the administration of 10-deazaaminopterin, a nontrivial analogue of methotrexate, the current drug of choice for the treatment of leukemia in the clinic, as well as 10-alkyl derivatives of 10-deazaaminopterin. It is expected that these compounds will have a similar effect in humans.

Rheumatoid arthritis is an inflammation of the joints arising from infectious, metabolic, or constitutional causes, usually of unknown origin. It can result in serious restriction of movement and even invalidism. Since rheumatoid arthritis is a common disease that affects 2-3 million people in the United State alone, it poses a serious treatment problem. A substantial proportion of affected individuals will develop erosive joint disease and require surgical joint replacement, despite therapies including disease-modifying antirheumatic drugs such as gold complexes, penicillamine, antimalarials, and methotrexate. In some patients with intractable rheumatoid arthritis, immunosuppressive agents including azathioprine, methotrexate, cyclophosphamide, and combinations of these drugs have been proven beneficial. However, the potential side effects of some of these drugs, including bone marrow toxicity and neoplasia, have limited their frequency of use and the dose that is given.

The disease is one of a number of forms of proliferative disease, and the development of drugs for amelioration or curing the disease has occupied the attention of research organizations for many years, until most recently without appreciable success.

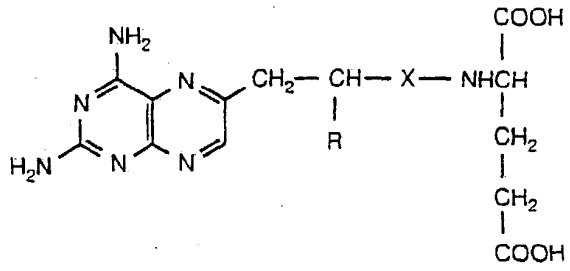
The antifolic acid drug, methotrexate, has been used as an antitumor agent since 1955. Its cytotoxic action in tumors is related to its ability to inhibit (essentially irreversibly) the key enzyme, dihydrofolate reductase, required for biosynthesis of tetrahydrofolic acid. Tetrahydrofolate is a vital component in one-carbon metabolism in cells, being required for biosynthesis of purine and pyrimidine nucleosides of the DNA and RNA. The drug is a powerful cytotoxic agent whose principal toxicities occur with liver, kidney, and mucosal tissue. Liver toxicity is the paramount concern for use in chronic therapy in a disease such as arthritis.

The ability of methotrexate to affect the inflammatory conditions of rheumatoid arthritis may be linked to its cytotoxic behavior. This may be in the nature of immune suppression and could involve attack on inflammatory phagocytic cells such as macrophages or neutrophils and T-helper cells in the synovial region. Very few methotrexate analogs have been evaluated against arthritis in animals, and there is no clear indication whether the antiarthritic properties are directly proportional to cytotoxicity. Galivan et al., Chem. Biol. Pteridines, DeGuyter, Berlin, 847 (1986), showed that adjuvant arthritis and streptoccocal cell wall arthritis in rats responded to doses of methotrexate relative to those used in man for treatment of rheumatoid arthritis. They also found that timing of dosage was most important for reduction of inflammation. Both methotrexate and aminopterin were found to inhibit inflammation, but other antifolate compounds that did not possess a 2,4-diaminopyrimidine unit or a benzoylglutamate side chain were ineffective.

What is needed is an effective treatment for inflammatory diseases, such as rheumotoid arthritis, which exhibits relatively low toxicity compared to current treatments.

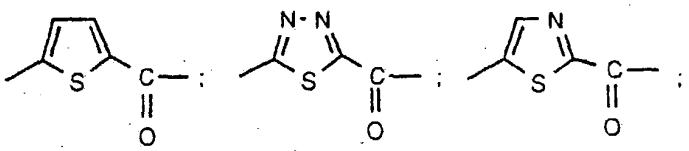
Description of the Invention

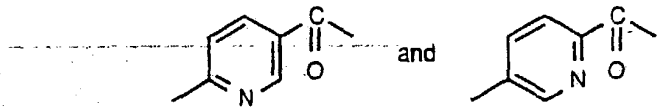
In accordance with the present invention, heteroaroyl-10-deazaaminopterin compounds are provided having the structure of Formula I:



I

wherein X is one of





and R is hydrogen or alkyl, alkenyl, or alkynyl having from one to about eight, preferably from three to five, carbon atoms.

Exemplary R alkyl include methyl, ethyl, propyl, iso-propyl, butyl, isobutyl, sec-butyl, tert-butyl, amyl, iso-amyl, sec-amyl, tert-amyl, hexyl, iso-hexyl, heptyl, iso-heptyl, octyl, iso-octyl, 2-ethyl hexyl, and tert-octyl.

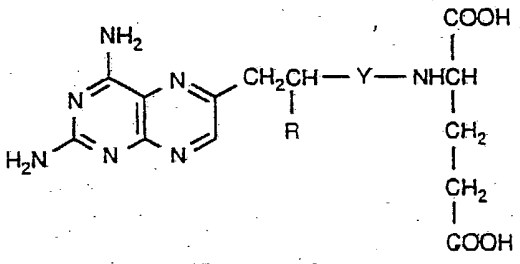
Exemplary R alkenyl include allyl, 1-propenyl, crotyl (2-butenyl), 2-pentenyl, 4-pentenyl, 2-hexenyl, 5-hexenyl, 3-isopropenyl, 3-isobutenyl, and 4-octenyl.

Exemplary R alkynyl include propargyl, 2-butynyl, 3-butynyl, 4-pentynyl, 5-hexynyl, and 7-octynyl.

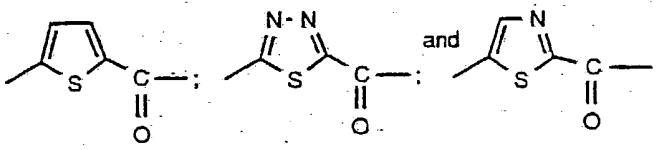
The invention also provides a method of treating arthritis and other proliferative diseases, which comprises administering to a warm-blooded animal having an inflammation of the joints or other evidence of the disease, a therapeutic nontoxic amount of a heteroaroyl-10-deazaaminopterin compound as defined hereinabove, as such or in the form of a pharmaceutically acceptable salt thereof. These salts are formed with one or more free NH_2 groups and/or COOH groups of the heteroaroyl-10-deazaaminopterin compound.

These compounds are believed to be novel, and in addition, are effective in the treatment of arthritis.

One subclass of thienyl compounds and thienyl analogues within the scope of the invention is defined by Formula II:

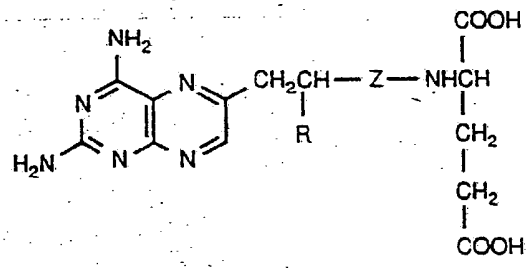


wherein Y is one of

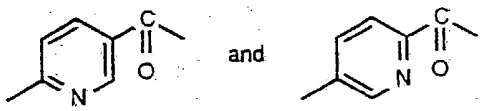


and R is hydrogen or alkyl, alkenyl, or alkynyl having from three to about eight, preferably from three to five, carbon atoms.

A subclass of pyridyl compounds within the scope of the invention is defined by Formula III:



wherein Z is one of



and R is hydrogen or alkyl, alkenyl, or alkynyl having from three to about eight, preferably from three to five, carbon atoms.

Exemplary heteroaroyl-10-deazaaminopterin compounds falling within Formula I are shown in the following Table I.

Table I

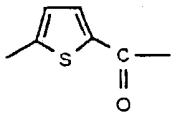
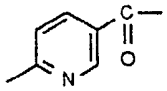
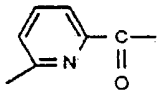
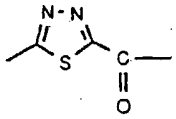
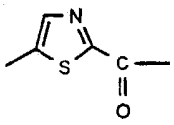
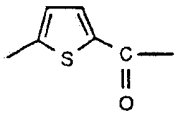
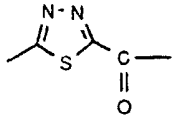
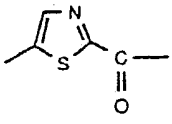
Compound No.	R_1	R_2
1	H	
2	C_2H_5	
3	H	
4	C_2H_5	
5	H	
6	C_2H_5	
7	H	
8	C_2H_5	
9	H	
10	C_2H_5	
11	CH_3	
12	CH_3	
13	CH_3	

Table I

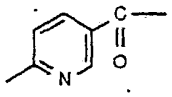
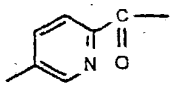
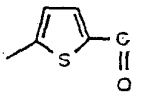
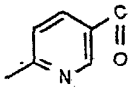
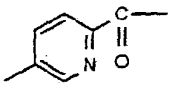
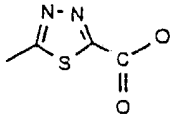
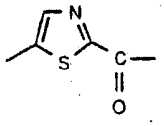
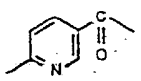
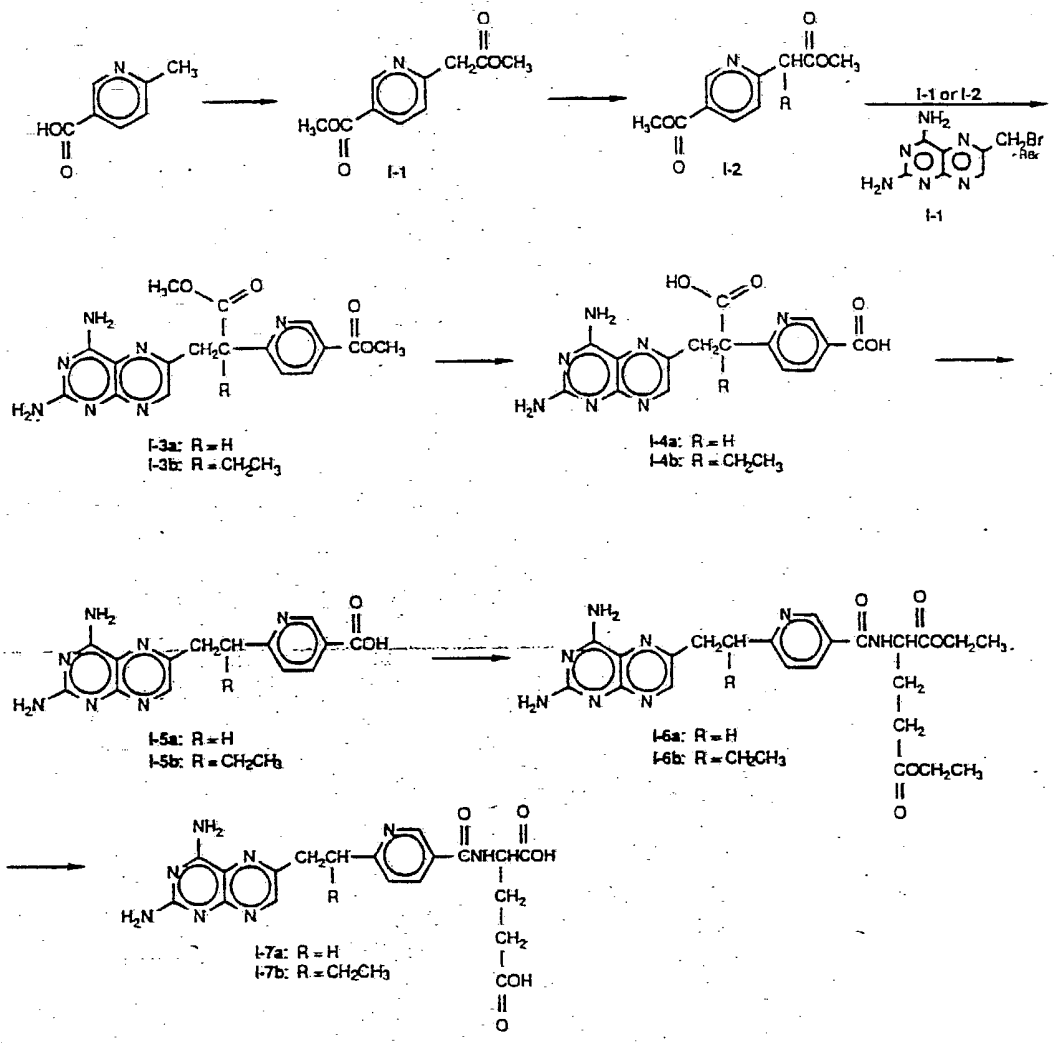
Compound No.	R_1	R_2
14	CH_3	
15	CH_3	
16	C_3H_7	
17	*i*-C_3H_7	
18	n-C_4H_9	
19	CH_2=CH-CH_2-	
20	CH≡CCH_2	
21	C_5H_{11}	
22	C_8H_{17}	
23	C_3H_7	
24	*i*-C_3H_7	
25	n-C_4H_9	
26	CH_2=CH-CH_2-	
27	CH≡CCH_2	
28	C_5H_{11}	
29	C_8H_{17}	
30	C_3H_7	
31	*i*-C_3H_7	
32	n-C_4H_9	
33	CH_2=CH-CH_2-	
34	CH≡CCH_2	
35	C_5H_{11}	
36	C_8H_{17}	

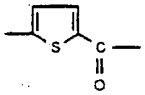
Table I

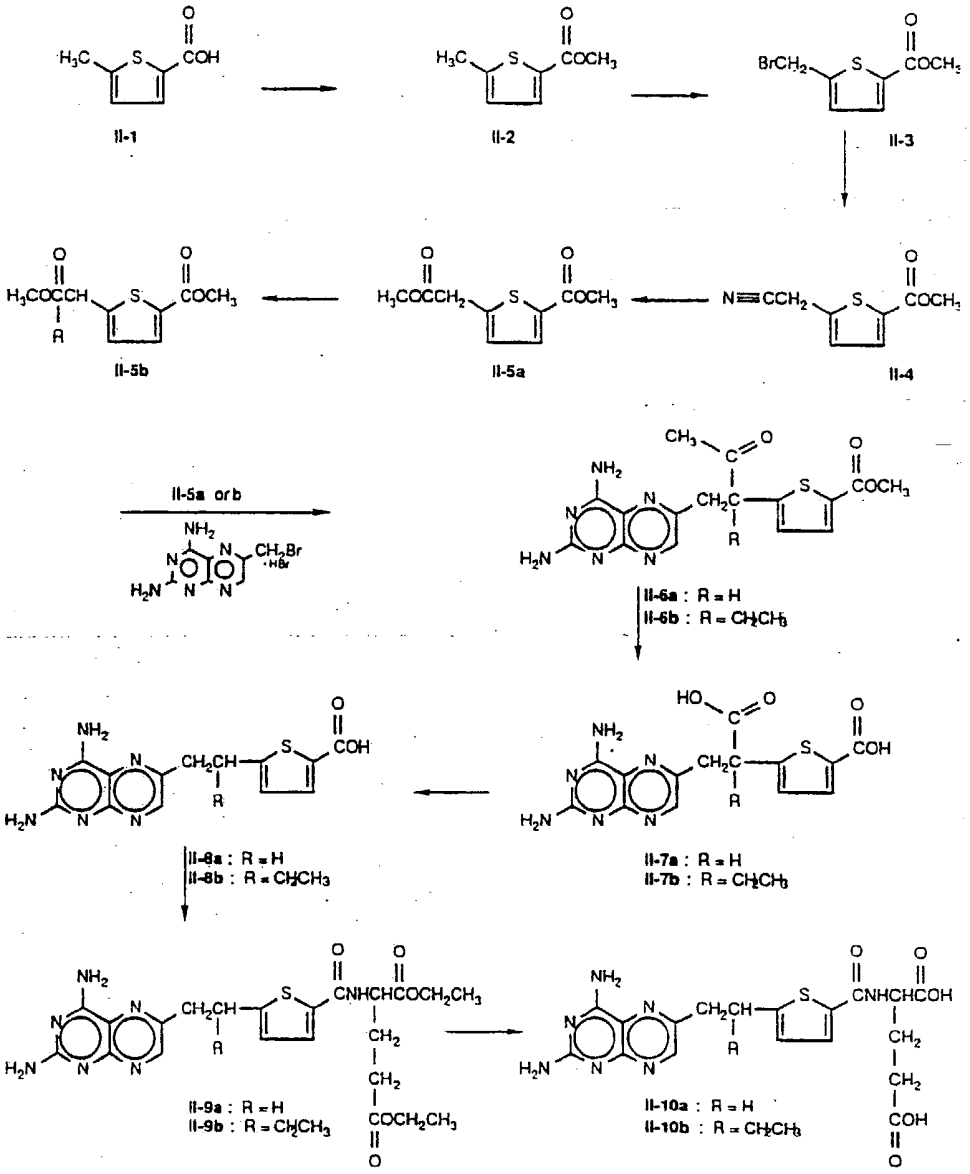
Compound No.	R_1	R_2
37	C_3H_7	
38	*i*-C_3H_7	
39	n-C_4H_9	
40	CH_2=CH-CH_2-	
41	CH≡CCH_2	
42	C_5H_{11}	
43	C_8H_{17}	
44	C_3H_7	
45	*i*-C_3H_7	
46	n-C_4H_9	
47	CH_2=CH-CH_2-	
48	CH≡CCH_2	
49	C_5H_{11}	
50	C_8H_{17}	

The compounds of Formula I, wherein X is  , may be synthesized by the following procedure, designated Procedure I:

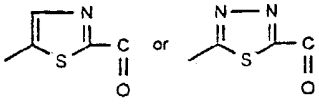


Procedure I

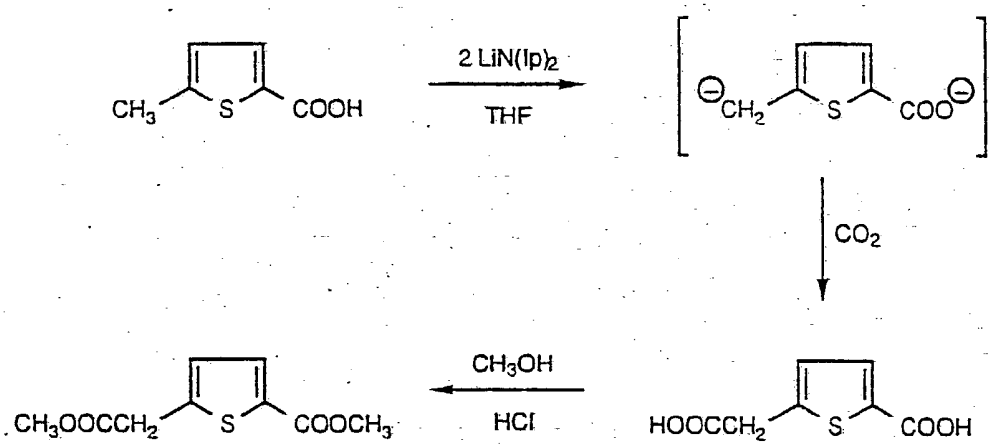
The following procedure, Procedure II, may be used to synthesize the compounds of Formula I wherein X is  (thiophene):



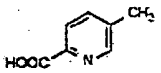
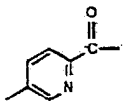
Procedure II: Thiophene analogs of 10-deazaaminopterins

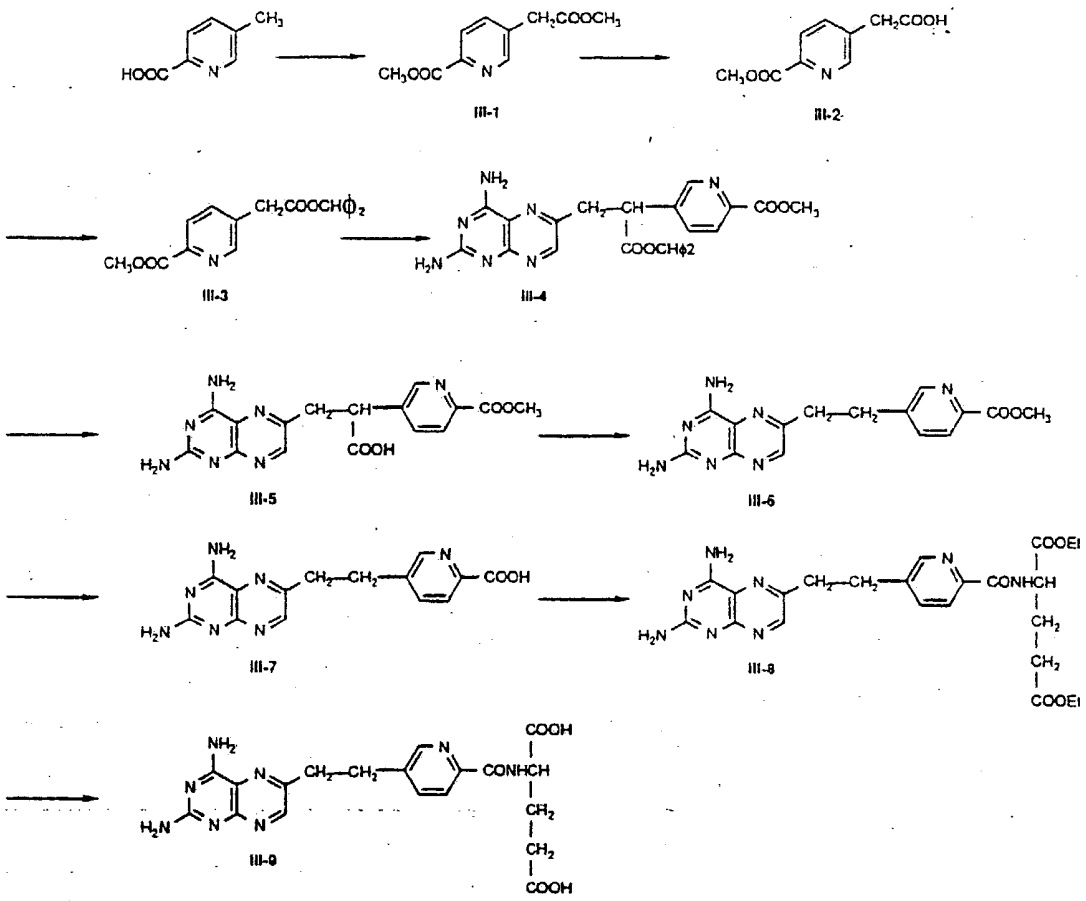
The above procedure may be applied to cases wherein X is  by substituting the corresponding thiazole or thiadiazole analogs.

A simpler and improved process for the thiophene dimethyl ester (step 11-5a in Procedure II) is as follows:



II-5a

A related procedure, Procedure III, substitutes  as a starting point wherein X is  in Formula I. The synthetic process differs slightly from Procedures I and II at intermediate steps **III-2** to **III-5** because it is necessary to have the pyridine carboxylate protected as an ester to prevent its decarboxylation in the steps **III-5** to **III-6**.



Procedure III

The following Examples represent preferred embodiments of the application of the synthesis of Procedures I-III to the preparation of compounds 1, 2, 3, 4, and 5 of Table I.

Examples A & B: Synthesis Of Compounds 1 And 2, Table I, By Procedure II

Example A

5-Bromomethyl-2-carbomethoxythiophene (II-3)

This compound was prepared from 5-methylthiophene-2-carboxylic acid by the method of Gogte et al, Tetrahedron, 23, 2443-51 (1967).

5-Cyanomethyl-2-carbomethoxythiophene (II-4)

A mixture of **II-3** (18.0 g, 76.6 mmol), sodium cyanide (15.0 g, 0.31 mmol) benzyltrimethylammonium chloride (1.75 g, 9.4 mmol) dichloromethane (75 mL), and water (75 mL) was stirred rapidly for 16 h. The mixture was then separated. The organic layer was treated

with water (75 mL), then sodium cyanide (15.0 g, 0.31 mmol), then benzyltrimethylamonium chloride (1.5 g, 8.0 mmol). This mixture was again rapidly stirred for 24 h. The organic layer was removed, dried over magnesium sulfate, and concentrated. The residue was chromatographed on 250 g of flash silica gel (20% ethyl acetate in hexanes eluent) to give the product as a yellow crystalline solid, 4.53 g (33%). Analysis gave the following results. NMR ($CDCl_3$) d 7.66 (d, 1H, C_3-H); 7.03 (d, 1H, C_4-H); 3.83 (d, 5H, CH_3 + CH_2); mass spectrum m/e 196 (M+ H); TLC (10% ethyl acetate in hexanes on silica gel plates); R_f - 0.3.

2-Carbomethoxythiophene-5-acetic Acid Methyl Ester (II-5a)

A solution of **II-4** (0.5 g, 2.7 mmol) and water (0.2 g) in methanol (7.5 mL) was treated dropwise with concentrated sulfuric acid (1.5 mL). This solution was stirred under argon at 65°C for 4 days. The pale yellow solution was poured onto ice-water (50 mL) and extracted with ether (2 x 50 mL). The organic extracts were combined and washed with water, saturated sodium bicarbonate then water again, dried over magnesium sulfate; and concentrated to a clear, colorless oil that solidified to a white, waxy solid (0.4 g, 68%). Analysis gave the following results. ($C_9H_{10}O_4S$) C, H,N, NMR ($CDCl_3$): d 7.61 (d, 1H, 3-H); 6.90 (d, 1H, 4-H); 3.87 (m, 5H, $ArCOO\underline{CH_3}$ + CH_2); 3.82 (s, 3H, $CH_2COO\underline{CH_3}$). TLC (10% ethylacetate in hexane on silica gel) R_f=0.4.

b-[3-(2,4-Diaminopyrimido [4,5-b]pyrazin-6-yl)]-a-carbomethoxy-5ethyl-2-carbomethoxythiophene (II-6a)

A suspension of sodium hydride (0.84 g, 17.5 mmol of sodium hydride) in 15 mL of dry dimethyl formamide was cooled to 0°C. A solution of the diester (**II-5a**, 3.73 g., 17.4 mmol) in 15 mL of dry dimethyl formamide was added dropwise. The resulting mixture was stirred at 0°C for 1 h then cooled to -30°C and treated with a solution of 2,4 diamino-6-bromomethyl pteridine hydrobromide (16.1 mmol) in 40 mL of dry dimethyl formamide. The resulting mixture was stirred for 2.5 h while rising to room temperature, then neutralized (pH = 7.5) by adding solid carbon dioxide. The mixture was concentrated under high vacuum, and the residue was washed with ether, then water, and dried under high vacuum to give the product as a yellow solid (1.98 g., 88%). Analysis gave the following results. Mass spectrum m/e 389 (M + H). NMR (d_6DMSO) d 8.58 (s, 1H, C_7-H); 7.60 (m, 3H, C_4-H + NH_2); 7.12 (d, 1H, C_3'-H); 6.61 (broad s, 2H, NH_2); 4.9 (t, 1H, C_{10}-H); 3.75 (s, 3H, C_2'-$COO\underline{CH_3}$); 3.63 (m, 5H, C_{10}-$COO\underline{CH_3}$ + C_9-H_2).

b-[3-2,4-Diaminopyrimido [4,5-b] pyrazin-6-yl)]-a-carboxy-5-ethylthiophene-2-carboxylic Acid (II-7a)

A solution of the diester (**II-6a** I, 1.96 g, 5.05 mmol) in 30 mL of 2-methoxy ethanol, water, and 30 mL of 2.5 molar sodium hydroxide was stirred for 1.5 h. The mixture was filtered, and the filtrate was neutralized (pH = 7) with acetic acid and concentrated under high vacuum. The residue was suspended in water (30 mL) and adjusted with acetic acid to pH = 5 to yield a precipitate. Filtration gave a tan solid that was digested in 95% ethanol. Filtration gave a tan solid that was washed with ether and dried *in vacuo*, yielding 1.31 g (77%) of product. Analysis gave the following results. HPLC (Novapak C18 column, 25% methanol in 0.1 molar NaH2PO4, pH 6.5) indicated 92.2% purity; NMR (d_6DMSO) d 8.51 (s, 1H, C_7-H); 7.55 (broad s, 2H, NH_2); 7.17 (d, 1H, 4'-H); 6.81 (d, 1H, 3'-H); 6.55 (broad s, 2H, NH_2); 4.40 (t, 1H, C_{10}-H); 3.15 (m, 2H, C_9-H_2).

b-[3-(2,4-Diaminopyrimido[4,5-b]pyrazin-6-yl)]-5-ethylthiophene-2-carboxylic Acid (II-8a)

A solution of the dicarboxylic acid (**II-7a**, 1.31 g, 3.64 mmol) in argon purged dimethylsulfoxide was placed in a 135°C oil bath for 45 min. The solution was then concentrated under high vacuum to a residue that was digested in ether. Filtration yielded a brown solid that was washed with ether and dried in vacuo at room temperature to give 1.31 g of crude product, which was suspended in water (75 mL) and treated dropwise to pH = 12 with ammonium hydroxide. The mixture was filtered and the filtrate adjusted to pH = 5 with acetic acid. Filtration gave a brown solid that was dried *in vacuo*, yielding 0.97 g product (84%). Analysis gave the following results. HPLC (see above conditions) indicated 86% purity. Anal. Calcd. for $C_{13}H_{12}N_6O_2S \cdot H_2O$: C, 46.69%; H, 4.22%; N, 25.13%. Found: C, 46.80%; H, 4.01%; N, 24.82%.

b-[3-(2,4-Diaminopyridimido[4,5-b]pyrazin-6-yl)]-5-ethylthiophene-2-carboxyl-glutamic Acid Diethyl Ester (II-9a)

A solution of the carboxylic acid (**II-8a**, 0.7g, 2.2 mmol) in dry dimethyl formamide (40 mL) was treated with triethyl amine (2.1 g, 21.0 mmol) and stirred at room temperature for 1.25 h. Isobutyl chloroformate (0.63 g, 4.6 mmol) was added, and the mixture was stirred for 1 h. L-Glutamic acid diethyl ester hydrochloride (1.1 g, 4.6 mmol) was added, and the mixture was stirred at room temperature for 2 h. Isobutyl chloroformate (0.32 g, 2.3 mmol) was then added, and the mixture was stirred for 1 h. L-glutamic acid diethyl ester hydrochloride (0.55 g, 2.3 mmol) was added, and the mixture was stirred for 1 h. Isobutyl chloroformate (3.2g, 2.3 mmol) was added, and the mixture was stirred at room temperature for 1 h. L-Glutamic acid diethyl ester hydrochloride (0.55 g, 2.3 mmol) was added, and the mixture was stirred at room temperature overnight. Concentration under high vacuum gave a dark residue that was washed repeatedly with ether. The residue was then washed with dilute ammonium hydroxide, then

water. The resultant orange solid was dried *in vacuo*. Chromatography on flash silica gel (2.5% methanol in chloroform) gave the product as a yellow powder, 0.32 g (32%). Analysis gave the following results. NMR (d_6DMSO + $CDCl_3$) d 8.5 (s, 1H, C_7-H); 8.31 (d, 1H, NHC); 7.6 (d, 1H, 4'-H); 6.80 (d, 1H, 3'-H); 6.32 (broad s, 2H, NH_2); 4.54 (m, 1H, CHNH); 4.18 (m, 4H, 2 x OCH_2); 3.28 (m, C_9-H_2); 2.42 (t, 2H, glu C_4-H_2); 2.13 (m, 2H, glu C_3-H_2); 1.28 (m, 6H, 2 x CH_3CH_2).

b-[3-(2,4-Diaminopyrimidino[4,5-b]pyrazin-6-yl)]-5-ethylthiophen-2-carboxyl-glutamic Acid (II-10a, Compound No. 1)

A mixture of the diester (**II-9a**, 0.26 g., 0.5 mmol) in 2-methoxyethanol (5 mL) was treated with water (5 mL) and 10% sodium hydroxide (5 mL). The mixture was stirred for 1 h then adjusted to pH = 5.5 with 2-N hydrochloric acid and concentrated under high vacuum. The residue was digested in water (5 mL) and the mixture was filtered. The resulting solid was washed with water and dried *in vacuo* at room temperature, giving 0.19 g of product (82%). Analysis gave the following results. HPLC (see above conditions) shows 96.4% purity. UV (0,1N NaOH) 258 nm (28,310); 372 nm (6,737). NMR (d_6DMSO) d 8.67 (s, 1H, C_7-H); 8.50 (d, 1H, NHCH); 8.00 (broad s, 2H, NH_2); 7.65 (d, 1H, 4'-H); 6.90 (broad s, 3H, 3'-H + NH_2); 4.30 (m, 1H, CHNH); 3.42 (m, C_9-H_2 + C_{10}-H_2); 2.35 (t, 2H, glu-C_4-H_2); 1.95 (m, 2H, glu C_3-H_2). Mass spectrum (DCl-NH_3) m/e 734 (TMS_4) (M+ H). Anal. Calcd. for $C_{18}H_{19}N_7O_5S \cdot 2H_2O$: C, 44.90%; H, 4.81%; N, 20.36%. Found: C, 44.68%; H, 4.39%; N, 20.32%.

Example B

a-Ethyl-2-carbomethoxythiophene-5-acetic Acid Methyl Ester (II-5b)

A suspension of sodium hydride (0.59 g, 12.2 mmol of sodium hydride) in 20 mL of dry dimethyl formamide was cooled to 0°C. A solution of **II-5a** (2.60 g, 12.2 mmol) in 20 mL of dry dimethyl formamide was added, and the reaction was stirred for an additional hour at 0°C. The reaction was cooled to -30°C and treated dropwise with a solution of ethyl iodide (1.9 g, 12.2 mmol) in dry dimethyl formamide, then stirred for 2.5 h at 20°C. The solution was neutralized (pH = 8) by adding solid carbon dioxide, then concentrated under high vacuum. The residue was digested in ether (250 mL) and filtered. The filtrate was washed with water, then saturated sodium bicarbonate, then 10% sodium bisulfate, then water again. The organic layer was dried on magnesium sulfate and concentrated. The residue was chromatographed on flash silica gel (ethyl acetate/hexanes eluent) to yield the product as a clear, colorless oil, 1.7g (58%). Analysis gave the following results. TLC (10% ethyl acetate in hexanes on silica gel plate), Rf = 0.35. NMR ($CDCl_3$) d 7.59 (d, 1H, Ar 3-H); 7.20 (d, 1H, Ar 4-H); 3.81 (m, 7H, 2 x $O\underline{CH_3}$ + Ar$\underline{CH}$); 2.06 (m, 2H, $\underline{CH_2}CH_3$); 0.95 (t, 3H, $\underline{CH_3}CH_2$).

b-[3-(2,4-Diaminopyrimido[4,5-b]pyrazin-6-yl)]-a-carbomethoxy-a-ethyl-5-ethyl-2-carbomethoxythiophene (II-6b)

A mixture of sodium hydride (0.4 g, 8.3 mmol of sodium hydride) in dry dimethyl formamide (25 mL) was cooled to 0°C and treated dropwise with a solution of the diester (**II-5b**, 2.0 g, 8.3 mmol) in dry dimethyl formamide (25 mL), stirred at 0°C for 1h, then cooled to -30°C. A solution of 2,4-diamino-6-bromomethylpteridine hydrobromide (2,7 mmol) in dimethylformamide (50 mL) was added dropwise, maintaining a -25°C internal temperature, then stirred an additional 2.5 h while warming to room temperature. The reaction was then adjusted to pH = 7 with carbon dioxide and concentrated under a high vacuum to yield a yellow residue that was stirred in ether. Filtration gave a yellow solid which was washed with water and dried *in vacuo* to yield 0.97 g of product (85%). Analysis gave the following results. NMR (d_6DMSO) d 8.35 (s, 1H, C_7-H); 7.78 (broad s, 1H, NH); 7.65 (d, 1H, C_4'-H);7.17 (d, 1H, C_3'-H); 6.65 (broad s, 2H, NH_2); 6.52 (broad s, 1H, NH); 3.77 (s, Ar$COOO\underline{CH_3}$); 3.68 (s, $CCOO\underline{CH_3}$); 2.06 (m, 2H, $\underline{CH_2}CH_3$); 0.76 (t, 3H, $\underline{CH_3}CH_2$). Mass spectrum (EI) m/e 416 (M + H).

b-[3-(2,4-Diaminopyrimidino[4,5-b]pyrazin-6-yl)]-a-carboxy-a-ethyl-5-ethylthiophene-2-carboxylic Acid (II-7b)

A mixture of the diester (**II-6b**, 0.95 g, 2.3 mmol) in 2-methoxyethanol (15 mL), water (15 mL), and 15 mL of 10% sodium hydroxide (15 mL) was stirred for 3.5 h. The solution was adjusted to pH = 5 by dropwise addition of 2N HCl, and the resulting mixture was concentrated under high vacuum. The residue was digested in water, then filtered to yield a cream-colored solid that was washed with water, then dried *in vacuo* at room temperature, giving 0.51 g (58%) of product. HPLC (see above conditions) showed 97% purity.

b-[3-(2,4-Diaminopyrimido [4,5-b]pyrazin-6-yl]-a-ethyl-5-ethylthiophene-2-carboxylic Acid (II-8b)

A solution of the dicarboxylic acid (**II-7b**, 0.22 g, 0.57 mmol) in dry dimethylsulfoxide (10 mL) was heated to 125°C for 30 min. The amber solution was then concentrated under high vacuum, and the residue was washed thoroughly with ether, then suspended in water (10 mL). Sufficient ammonium hydroxide was added to bring about solution, then adjusted to pH = 5 with hydrochloric acid and filtered. The resulting tan solid was washed with water, then dried *in vacuo* , yielding 0.14 g (70%). HPLC (Novapak C18 radial compression column, 25% methanol in 0.1N monobasic sodium phosphate) indicated 90.5% purity. Analysis gave the following results. Quant UV (0.1N NaOH) 256 nm (28.546), 372 (7,300). Mass spectrum (DCI-NH_3) 561 (TMS_3) = 345 (M + H). Anal. Calcd. for $C_{15}H_{16}N_6O_2S \cdot 0.6\,H_2O$: C, 50.72%; H, 4.88%; N, 23.66%. Found: C, 50.54%; H, 4.94%; N, 23.91%.

N-[a-Ethyl-b-(2,4-diamino-[4,5-b]-pyrazin-6-yl)-5-ethylthiophene-2-carbonyl]-glutamic Acid Diethyl Ester (II-9b)

A mixture of the carboxylic acid (**II-8b**, 0.99 g, 2.9 mmol) and triethyl amine (2.7 g, 26.7 mmol) in dry N,N-dimethylformamide (50 ml) was stirred at room temperature for 1 h, then treated with isobutyl chloroformate (0.81 g, 5.9 mmol). The mixture was stirred for 1 h, treated with L-glutamic acid diethyl ester hydrochloride (1.42 g, 5.9 mmol), and stirred at room temperature for 2 h. Isobutyl chloroformate (0.41 g, 3.0 mmol) was added and the mixture was stirred at room temperature for 1 h. L-glutamic acid diethyl ester hydrochloride (0.72 g, 3.0 mmol) was added and the mixture was stirred at room temperature for 1 hr. Isobutyl chloroformate (0.41 g, 3.0 mmol) was added and the mixture was stirred for 1 h. L-glutamic acid diethyl ester hydrochloride (0.42 g, 3.0 mmol) was added and the reaction mixture was stirred at room temperature for 16 h. The mixture was concentrated under high vacuum. The yellow solid was washed with water then dried *in vacuo*. Chromatography on flash silica gel (2% methanol in chloroform eluent) gave the product as a yellow foam in 20% yield (0.3g).

Analysis gave the following results. NMR ($CDCl_3$): d = 0.90 (t, 3 H C_{10}-CH_2-$\underline{CH_3}$); 1.30 (m, 6 H, 2 ¥ $OCH_2\underline{CH_3}$); 2.17 (m, 2 H, glu C_3-H_2); 2.47 (m, 2 H, glu C_4-H_2); 3.20 (m, 3 H, C9-H2 + C_{10}-H); 4.16 (m, 4 H, 2 ¥ $O\underline{CH_2}$); 4.75 (m, 1 H, $\underline{CH}NH$); 5.45 (broad s, NH); 6.55 (m, 1 H, C_3-H); 6.95 (m, 1 H, $\underline{NH}CH$); 7.30 (d, 1 H, C_4-H); 8.41 (d, 1 H, C_7H). Anal. Calcd. for $C_{24}H_{31}N_7O_5S \cdot 0.7\ H_2O$: C, 53.16%; H, 5.93%; N, 18.08; O, 16.82%. Found: C, 53.43%; H, 5.79; N, 17.73%; O, 16.90%.

N-[a-Ethyl-b-(2,4-diamino-[4,5-b]-pyrazin-6-yl)-5-ethylthiophene-2-carbonyl]-glutamic Acid (II-10b, Compound No. 2)

A solution of the diester (**II-9b**, 0.55 mmol) in 2-methoxyethanol (10 ml) was treated with 10% sodium hydroxide (5 ml) and water (5 ml). After stirring for 75 min the solution was neutralized (pH = 5) with 2 molar hydrochloric acid and concentrated under high vacuum. The residue was treated with water and the mixture filtered. The yellow solid was dried *in vacuo*, yielding 0.15 g of product (57%). HPLC (Novapak C_{18} radial compression column, 25% methanol in 0.1 molar monobasic sodium phosphate, pH 6.5), 96.9% purity. Analysis gave the following results. UV (0.1 N, NaoH) 256 nm (28, 139), 371 (6, 810); mass spectrum (DCl-NH_3) m/e 762 (TMS_4 M+H).

Examples C & D: Synthesis Of Compounds 3 And 4, Table I, By Procedure I

Example C

5-Carbomethoxy-2-pyridylacetic Acid Methyl Ester (I-1)

Freshly distilled diisopropylamine (7.4 g, 73 mmol) in dry tetrahydrofuran (100 mL) was cooled under argon to 0°C then treated dropwise with n-butyl lithium in hexanes (50 mL of a 1.6-M solution) and stirred at 0°C for 1 h. The lithium diisopropyl amide solution was added dropwise over 45 min to a -25°C mixture of 6- methylnicointinic acid (4.0 g., 29 mmol) and hexamethylphosphorous triamide (5.23 g) in dry tetrahydrofuran. The temperature of the red solution was allowed to rise to 0°C whereupon stirring was continued for 2 h. Carbon dioxide was bubbled through the 0°C solution, resulting in a yellow precipitate. The mixture was allowed to rise to room temperature and was stirred for 16 h. Filtration gave a yellow solid that was suspended in methanol (50 mL) and the mixture was cooled to 0°C. Saturated methanolic HCl was added, and the solution was stirred at room temperature for 72 h. Concentration in vacuo gave a residue that was partitioned between ether and saturated sodium bicarbonate. The

ether layer was washed with water, dried over magnesium sulfate, and concentrated to an orange oil. Chromatography on flash silica gel (5% ethyl acetate in hexanes) gave the product as a yellow solid, 1.84 g (30%). Analysis gave the following results. M.p. 56-57°; NMR ($CDCl_3$): d 9.10 (m, 1H, 6-H); 8.21 (m, 1H, 4-H); 7.33 (m, 1H, 3-H); 3.84 (m, 8H, $\underline{CH_2}COO\underline{CH_3}$ + $ArCOO\underline{CH_3}$). Anal. Calcd. for $C_{10}H_{11}NO_4$: C, 57.41%; H, 5.30%; N, 6.70%. Found: C, 57.53%; H, 5.33%; N, 6.54%.

a-Ethyl-5-carbomethoxy-2-pyridylacetic Acid Methyl Ester (I-2)

A 0°C suspension of sodium hydride (1.14 g, 50% in oil, 0.57g of sodium hydride, 23.8 mmol) in dry dimethyl formamide was treated dropwise with a solution of **I-1** (4.98 g, 23.8 mmol) in dry dimethyl formamide (15 mL). This mixture was stirred at 0°C for 1h, then cooled to -30°C. A solution of ethyl iodide (3.72 g, 23.8 mmol) in dry dimethyl formamide (50 mL) was added dropwise, maintaining a -25°C reaction temperature, then stirred for 2 h at room temperature. The reaction was neutralized (pH = 8) by adding solid carbon dioxide, then concentrated under high vacuum. The residue was partitioned between ether and water. The organic layer was washed with 10% sodium bicarbonate, 10% sodium bisulfite, and water. The organic layer was dried over magnesium sulfate and concentrated to a pale brown oil. Chromatography on flash silica gel (5% ethyl acetate in hexanes) gave the product as a yellow oil (2.86 g, 51%) that was pure by TLC (10% ethyl acetate in hexanes on silica gel). Analysis gave the following results. NMR ($CDCl_3$) d 9.13 (m, 1H, 6-H); 8.26 (m, 1H, 4-H); 7.39 (m, 1H, 3-H); 3.83 (m, 7H, 2 X OCH_3 + a-CH); 2.10 (m, 2H, $\underline{CH_2}CH_3$); 0.87 (t, 3H, $\underline{CH_3}CH_2$). Anal. Calcd. for $C_{12}H_{15}NO_4$: C, 60.75%; H, 6.37; N, 5.90. Found: C, 60.63%; H, 6.38%; N, 5.89%.

3-(2,4-Diaminopyrimido[4,5-b]pyrazin-6-yl)-2-(3-carbomethoxypyrid-6-yl)-propionic Acid Methyl Ester (I-3a)

To a 0° C suspension of sodium hydride (0.69 g of 50% sodium hydride in oil, 14.3 mmol) in a dry dimethyl formamide (10 mL) was added dropwise a solution of **I** (3.0 g, 14.3 mmol) in dry dimethyl formamide (10 mL). The mixture was stirred at 0° C for 30 min, then cooled to -30°C. A solution of 2,4 diamino-6-bromomethylpteridine hydrobromide (4.8 mmol) in dry dimethyl formamide (30 mL) was added dropwise over 40 min. The reaction was stirred for 2.5 h at 10°C, then adjusted to pH 8 by adding dry ice. Concentration under high vacuum gave a residue that was washed with ether, then water. Drying *in vacuo* at room temperature gave the product as a yellow solid, 1.8 g (99%). Anal. Calcd. for $C_{17}H_{17}N_7O_4 \cdot 1.7\ H_2O$: C, 49.32%; H, 4.96%; N, 23.68%. Found: C, 49.56%; H, 4.21%; N, 23.25%.

b-(2,4-Diamino-[4,5-b]pyrazin-6-yl)-6-ethylnicotinic Acid (I-5a)

A solution of the diester (I-3a, 1.8 g, 4.7 mmol) in 2-methoxyethanol (20 mL), water (20 mL), and 10% sodium hydroxide (20 mL) was stirred for 2.5 h, then diluted with water (40 mL). The reaction was adjusted to pH 6 with glacial acetic acid. The cream-colored precipitate was collected, washed with water, and dried to yield 1.61 g of product (97%); HPLC (Novapak C_{18} radial compression column, 25% methanol in 0.1 molar monobasic sodium phosphate, pH 6.5), 95.3% purity.

A mixture of the dicarboxylic acid (I-4a, 0.5 g, 1.4 mmol) in dry argon-purged dimethyl sulfoxide (40 mL) was heated to 110°C for 25 min, then concentrated under high vacuum. The residue was suspended in water (40 mL), and sufficient ammonium hydroxide was added to produce a solution. The solution was adjusted to pH 5 by dropwise addition of glacial acetic acid, then the precipitate was collected. The resulting yellow solid was washed with water and dried to yield 0.4 g product (94%). HPLC (see above conditions) shows 92% purity: Analysis gave the following results. Mass spectrum (EI) m/e 527 (TMS_3) 311. Anal. Calcd. for $C_{14}H_{13}N_2O_2 \cdot 2.0\,H_2O$: C, 48.41%; H, 4.93; N, 28.23. Found: C, 48.95; H, 4.89; N, 27.79.

N-[b-(2,4-Diaminopyrimido-[4,5-b]-pyrazin-6-yl)-6-ethylnicotinoyl]-glutamic Acid Diethyl Ester (I-6a)

A mixture of the carboxylic acid (I-5a, 0.4 g, 1.25 mmol) in dry dimethyl formamide was treated with triethyl amine (1.2 g, 11.8 mmol). After being stirred for 1 h, the mixture was treated with isobutyl chloroformate (0.35 g, 2.6 mmol). The mixture was stirred for 1 h at room temperature and treated with L-glutamic acid diethyl ester hydrochloride (0.62 g, 2.6 mmol). After 2 h, the mixture was treated with isobutyl chloroformate (0.18 g, 1.3 mmol). The mixture was stirred for 1 h and treated with L-glutamic acid diethyl ester hydrochloride (0.31 g, 1.3 mmol). After 1 h of stirring, isobutyl chloroformate (0.18 g, 1.3 mmol) was added. The mixture was stirred for 1 h. L-glutamic acid diethyl ester hydrochloride (0.31 g, 1.3 mmol) was added, and the mixture was stirred for 16 h. The mixture was concentrated under high vacuum. The residue was washed thoroughly with ether, then with water. The residue was crystallized from hot ethanol, giving yellow crystals (0.31 g, 50% theory). Analysis gave the following results. TLC (20% methanol in chloroform on silica gel plates) Rf = 0.2; mass spectrum (DCl-NH_3) m/e 497 (M + H). Anal. Calcd. for $C_{23}H_{28}N_8O_5 \cdot H_2O$: C, 53.68%; H, 5.87%; N, 21.77%. Found: C, 53.45%; H, 5.70%; N, 21.78%. NMR (d_6DMSO) d 8.90 (d, 1H, NHCO); 8.87 (d, 1H, pyr 6'-H), 8.61 (s, 1H, C_7-H); 8.10 (m 1H, pyr 4'-H); 7.70 (broad d. 1H, NH); 7.42 (d, 1H, pyr 3'-H); 6.65 (broad s, 2H, NH_2); 4.40 (m, 1H, $\underline{CH}N$); 4.05 (m, 4H, 2 X $O\underline{CH_2}$); 3.30 ($CH_2CH_2 + H_2O$); 2.45 (t, 2H, $\underline{CH_2}CO_2$); 2.05 (m, 2H, $\underline{CH_2}CH$); 1.70 (t, 6H, 2 X CH_3).

N-[b-(2,4-Diaminopyrimido-[4-5-b-]-pyrazin-6-yl)-6-ethylnicotinoyl-] glutamic Acid (I-7a) (Compound No. 3)

Diester (I-6a, 0.3 g, 0.6 mmol) was dissolved in 2-methoxyethanol (10 mL), 10% sodium hydroxide (5 mL) and water (4 mL) and stirred at room temperature for 2.5 h. The solution was then diluted with water (20 mL), adjusted to pH = 6 with acetic acid, and filtered. The resulting yellow solid was washed with water and dried to give the product as a fine powder (0.19 g, 71%). HPLC (see above conditions) shows 95% purity. Analysis gave the following results. Mass spectrum (DCl-NH_3) m/e 729 (TMS_4M + H) = 440; UV (0.1N NaOH) 258 nm (25,000) 275 sh (13, 900), 371 (6,600). Anal. Calcd. for $C_{19}H_{20}N_8O_5 \cdot 2.25\ H_2O$: C, 47.44%; H, 5.14%, N, 23.30%. Found: C, 47.04%; H, 4.64%; N, 23.64%.

Example D

3-(2,4-Diaminopyrimido[4,5-b]-pyrazin-6-yl)-2-(3-carbomethoxypyrid-6-yl)-2-ethylpropionic Acid Methyl Ester (I-3b)

A 0°C suspension of sodium hydride (0.56 g, 50% in oil, 11.8 mmol of sodium hydride) in dry methyl formamide (10 mL) was treated dropwise with a solution of the diester (I-2, 2.8 g, 11.8 mmol) in dry dimethylformamide (10 mL). The resulting mixture was stirred at 0°C for 1 h, then cooled to -30°C. A solution of 2, 4 diamino-6-bromomethylpteridine hydrobromide (3.9 mmol) in dry dimethyl formamide was added, maintaining a -25°C internal temperature. The reaction mixture was allowed to stir for 2 h as it rose to room temperature. The mixture was adjusted to pH 8 by adding dry ice. Concentration under high vacuum gave a residue that was washed with ether, then water. The resulting yellow solid was dried *in vacuo*, giving 1.26 g of product (78%). Analysis gave the following results. Mass spectrum m/e 412 (M+ H). NMR (d_6DMSO) d 9.04 (s, 1H, C_7-H); 8.23 (m, 2H, pyr 5'-H + pyr 4'-H); 7.45 (d, 1H, pyr 2'-H);6.62 (broad s, 2H, NH_2); 3.87 (s, 3H, $ArCOOCH_3$); 3.62 (m, 5H, C_{10}-$COOCH_3$ + C_9-H_2); 2.01 (m, 2H, $\underline{CH_2}CH_3$); 0.80 (t, 3H, $\underline{CH_3}CH_2$). Anal Calcd. for $C_{19}H_{21}N_7O_4 \cdot 1.5\ H_2O$: C, 52.04%; H, 5.51%; N, 22.36%. Found: C, 52.22%; H, 5.18%; N, 22.49%.

3-(2,4-Diaminopyrimido[4,5-b]pyrazin-6-yl)-2(3-carboxypyrid-6-yl)-2-ethylpropionic Acid (I-4b)

A solution of the diester (I-3b, 1.24 g, 3.0 mmol) in 2-methoxyethanol (20 mL), water (20 mL), and 10% sodium hydroxide (20 mL) was stirred for 15 h. The reaction was adjusted to pH 7 with glacial acetic acid, then concentrated under high vacuum. The residue was treated

with water (10 mL) and adjusted to pH 4 with 4N hydrochloric acid, and the precipitate was collected. The resulting tan solid washed with water and dried *in vacuo* to yield 0.31 g product (27%).

a-Ethyl-b-(2,4-diaminopyrimido-[4,5-b]-pyrazin-6-yl)-6-ethyl Nicotinic Acid (I-5b)

The dicarboxylic acid (**I-4b**, 0.31 g) was dissolved in dry dimethyl formamide (8 mL). The solution was allowed to stand at room temperature for 20 min. Concentration under high vacuum gave a residue that was washed with ether. The resulting tan solid was dried *in vacuo* to give the product in 99% yield. HPLC (see above conditions) showed the product to be of 90% purity.

N-[a-Ethyl-b-(2,4-diaminopyrimido-[4,5-b]-pyrazin-6-yl)-6-ethyl-nicotinoyl]-glutamic Acid Diethyl Ester (I-6b)

A mixture of the carboxylic acid (**I-5b**, 0.31 g, 0.75 mmol) and triethyl amine (0.73 g, 7.2 mmol) in dry dimethyl formamide (20 mL) was stirred at room temperature for 15 min. Isobutyl chloroformate (0.22 g, 1.6 mmol) was then added, and the mixture was stirred for 1 h. L-Glutamic acid diethyl ester hydrochloride (0.38 g, 1.6 mmol) was added, and the mixture was stirred for 2 h. Isobutyl chloroformate (0.11 g, 0.8 mmol) was added, and the mixture was stirred for 1 h. L-Glutamic acid diethyl ester hydrochloride (0.19 g, 0.8 mmol) was added, and the mixture was stirred at room temperature for 1 h. Isobutyl chloroformate (0.11 g, 0.8 mmol) was added, and the mixture was stirred for 1 h. L-Glutamic acid diethyl ester hydrochloride (0.19 g., 0.8 mmol) was added, and the mixture was stirred for 16 hr. The mixture was filtered and the filtrate concentrated under high vacuum. The residue was chromatographed on flash silica gel (5% methanol in chloroform eluent), giving the product as an orange glass (0.23 g, 48%). Analysis gave the following results. Mass spectrum (DCI-NH_3) m/e 525 (M + H); NMR ($CDCl_3$) d 9.01 (broad s, 1H, pyr 6'-H; 8.45 (broad s, 1H, 7-H); 7.97 (d, 1H, pyr 4'-H); 7.35 (broad s, 2H, NH_2); 7.08 (d, 1H, pyr 3'-H); 5.38 (broad s, 2H, NH_2); 4.75 (m, 1H, CHN): 4.19 (m, 4H, 2 X OCH_2); 3.32 (m, 3H, C_9-H_2 + C_{10}-H); 2.50 (m, 2H, C_{10}-CH_2); 2.23 (m, 4H, glu C_4-H_2 +glu C_3-H_2); 1.26 (m, (6H, 2 X OCH_2CH_3); 0.83 (t, 3H, C_{10}-CH_2CH_3).

N-[a-Ethyl-b-(2,4-diaminopyrimido-[4,5-b]-pyrazin-6-yl)-6-ethylnicotinoyl]-glutamic Acid (I-7b, Compound No. 4)

The diester (**I-6b**, 0.2g, 0.38 mmol) was dissolved in 2-methoxyethanol (6 mL) and 10% sodium hydroxide (1.6 mL) and stirred for 1 h at room temperature. The solution was adjusted to pH = 7 with acetic acid and concentrated under high vacuum. The residue was dissolved in water (7 mL) and acidified to pH 3 with 4-mol hydrochloric acid, then filtered. The resulting tan solid was washed with water and dried *in vacuo* to yield 70 mg of product (39%). HPLC (see

above conditions) showed 98.9% purity. Analysis gave the following results. Mass spectrum m/e 757 (TMS_4) = 467 (M + H); UV (0.1N NaOH) 256 nm (25,246) 367 (6562). Anal. Calcd. for $C_{21}H_{24}N_8O_5 \cdot 1.4\ H_2O$: C, 51.09%; H, 5.47%; N, 22.68%. Found: C, 51.12%; H, 5.29%; N, 22.55%.

Example E

2-Carbomethoxy-5-pyridylacetic Acid Methyl Ester (III-1)

The diester (**III-1**) was prepared in a manner similar to **I-1** from 5-methylpicolinic acid (10.0 g, 73 mmoles) resulting in an amber oil product in 49% yield. Analysis gave the following results. NMR ($CDCl_3$): d 8.63 (d, 1H, C_3-H); 8.15 (d, 1H, C_6-H); 7.81 (m, 1H, C_4-H); 4.02 (s, 3H $ArCOOCH_3$); 3.75 (s, 5H, CH_2COOCH_3).

2-Carbomethoxy-5-pyridylacetic Acid Benzhydryl Ester (III-3)

A solution of potassium hydroxide (1.39 g, 24.8 mmoles) in 90% methanol (100 mL) was treated with a solution of **III-1** (5.18 g, 24. 8 mmoles) in methanol (14 mL). After 2 h the solution was adjusted to pH 6.5 by hydrochloric acid addition. The solution was concentrated *in vacuo* to give a tan solid that was a mixture of both monoesters, the dicarboxylic acid and the starting diester. HPLC indicated the desired monoester (**III-2**) to represent 57% of the mixture.

The mixture (**III-2**) in chloroform (100 mL) was cooled to 0° C and treated dropwise with a solution of diphenyldiazomethane (5.27 g, 27.2 mmoles) in chloroform (50 mL). The resulting purple mixture was stirred at ambient temperature for 24 h. The solution was washed with saturated sodium bicarbonate and water. The organic layer was dried over magnesium sulfate and concentrated to a purple syrup. Crystallization from pentane gave the product as a white solid, 1.86 g (21% yield from **III-1**). Analysis gave the following results. NMR ($CDCl_3$): d 8.68 (m, 1H, C_3-H); 8.10 (d, 1H C_6-H); 7.75 (m, 1H, C_4-H); 7.30 (m, 10H, 2 ¥ C_6H_5); 6.90 (s, 1H, OCH); 4.05 (s, 3H, OCH_3); 3.81 (s, 2H, CH_2). Anal. Calcd. for $C_{22}H_{19}NO_4 \cdot 0.25\ H_2O$: C, 72.21; H, 5.37; N, 3.83. Found C, 72.43; H, 5.49; N, 3.69. TLC (40% ethyl acetate in hexanes on silica gel) showed a single spot at Rf 0.5.

3-(2,4-Diaminopyrimido[4,5-b\-pyrazin-6-yl)-2-(2-carbomethoxy-pyrid-5-yl)propionic Acid Benzhydryl Ester (III-4)

A 0° C suspension of sodium hydride (413 mg of 50% in oil, 8.6 mmoles) in dry N,N-dimethylformamide (20 mL) was treated dropwise with a solution of III-3 (3.11 g, 8.6 mmoles) in dry dimethylformamide (25 mL). The yellow-green mixture was stirred at 0° C for 2 h.

becoming a red solution. This was cooled to -25°C and treated. dropwise with a solution of 2,4-diamino-6-bromomethylpteridine hydrobromide (3.4 mmoles) in dry dimethylformamide (20 mL) with maintanence of the temperature at -25°C. The mixture was stirred at 22° C for 2.5 h and adjusted to pH 8 by addition of dry ice. Concentration under high vacuum gave a residue which was washed with ether and water. The yellow solid was dried *in vacuo* and chromatographed on flash silica gel (4% methanol in chloroform) to yield the product as a yellow powder 1.33 g (75% yield). Analysis gave the following results. NMR ($CDCl_3$): d 8.80 (m, 1H, C_7-H); 8.62 (s, 1H, C_3'-H); 8.10 (d, 1H, C_6'-H); 7.84 (m, 1H, C_4'-H); 7.20 (m, 12H, 2 ¥ C_6H_5 + NH_2); 6.80 (s, 1H, OCH); 5.20 (broad s, 2H, NH_2); 4.55 (m, 1H, C_{10}-H); 4.02 (s, 3H, OCH_3); 4.85 (m, 1H, C_9-H); 3.30 (m, 1H, C_9-H).

b-[3-(2,4-Diaminopyrimido[4,5-b]-pyrazin-6-yl)]-4-ethylpicolinic Acid Methyl Ester (III-6)

A mixture of the diester **III-4** (1.29 g, 2.4 mmoles) in dichloromethane (67 mL) was treated with 99% trifluoroacetic acid (33 mL). The yellow solution was kept at room temperature for 50 min then concentrated at room temperature under high vacuum. The residue was washed repeatedly with ether then dried *in vacuo* giving a bright yellow solid. This was suspended in water and neutralized to pH 8 with 1.5 M ammonium hydroxide. The mixture was concentrated under high vacuum giving a yellow solid, 0.99 g. HPLC showed the conversion to **III-5**.

A solution of the monocarboxylic acid, **III-5** (0.99 g crude) in 40 mL of dimethylsulfoxide, was stirred at 130° for 30 minutes. HPLC showed disappearance of the starting carboxylic acid (**III-5**) (retention time 4.4 minutes) and the desired monoester to be present (retention time 15.2 minutes). The solution was concentrated under high vacuum and the residue was washed with ether and water. The orange solid was collected and dried *in vacuo* at room temperature to afford 505 mg (64%). NMR ($CDCl_3$): d 8.60 (m, 2H, C_7-H, 6'-H); 8.10 (d, 1H, 3'-H); 7.85 (d, 1H, 5'-H); 7.20 (m, 3H, NH_2); 4.00 (s, 3H, OCH_3); 3.35 (s, 4H, CH_2CH_2).

b-[3-(2,4-Diaminopyrimido[4,5-b]-pyrazin-6-yl)]-4-ethyl Picolinic Acid (III-7)

A mixture of the ester **III-6** (0.49 g, 1.5 mmoles) in 2-methoxyethanol (5 mL) was treated with water (5 mL) then 10% sodium hydroade (2.5 mL). After stirring 45 min, the resulting red solution showed complete saponification by HPLC.

The solution was neutralized (pH 7.5) with hydrochloric acid and concentrated under high vacuum. The resulting residue was treated with water and stirred. Filtration gave 0.27 g of product as an orange solid (57%). HPLC showed 96% purity. Mass spectrum (EI) m/e 527 (TMS_3).

b-[3-(2,4)-Diaminopyrimido (4,5-b)-pyrazin-6-yl)]-4-ethylpicolinoyl]glutamic Acid Diethyl Ester (III-8)

A mixture of the carboxylic acid (**III-7**, 0.27 g, 0.87 mmol) and triethyl amine (822 mg, 8.12 mmol) in dry dimethyl formamide (15 mL) was stirred at room temperature for 15 min. Isobutyl chloroformate (0.23 mL, 1.78 mmole) was added and the mixture was stirred for 1 h. L-Glutamic acid diethyl ester hydrochloride (427 mg, 1.78 mmol) was added and the mixture was stirred for 2 h. The addition of isobutyl chloroformate and diethyl glutamate was repeated at one-half the initial quantities and the final mixture was stirred for 16 hours. After filtration, the filtrate was concentrated *in vacuo* and the residue was partitioned between water and chloroform. Chromatography of the chloroform soluble portion yielded 72 mg of the diester (**III-8**, 18%). Analysis gave the following results. NMR ($CDCl_3$): d 8.60 (d, 1H, C_7-H); 8.55 (d, 1H, NH); 8.43 (d, 1H, C_5'-H); 8.06 (d, 1H, C_2'-H); 7.70 (m, 1H, C_6'-H); 4.80 (m, 1H, CHNH); 4.20 (m, 4H, 2¥ OCH_2); 2.30 (m, 4H, glu CH_2CH_2); 1.30 (m, 6H, 2¥ OCH_2CH_3). Mass spectrum (EI) m/e 496.

b-[3-(2,4)-Diaminopyrimido[4,5-b]-pyrazin-6-yl]-4-ethylpicolinoyl]glutamic Acid (III-9, Compound No. 5)

The diester (**III-8**, 67 mg, 0.13 mmol) was dissolved in 2-methoxyethanol (2.3 mL) and 10% sodium hydroxide (2.2 mL) was added. The mixture was stirred for 2 h at room temperatuare. The solution was adjusted to pH 5-6 with acetic acid and evaporated *in vacuo*. The residue was dissolved in 2 mL of water and acidified to pH 3-4. The solid was collected, washed with water, and dried to leave 34 mg (58%). HPLC (see above conditions) shows 99.3% purity. UV (0.1 M NaOH) 371 (5,600); 257 (22,200). Mass spectrum (DCl-NH_3) m/e 729 (TMS_4).

The heteroaroyl-10 deazaaminopterin compound can be administered per se, or in association with a pharmaceutically acceptable diluent or carrier. The invention accordingly also provides a pharmaceutical composition in dosage unit form comprising from 0.1 to about 500 mg of heteroaroyl-10-deazaaminopterin compound, per dosage unit, together with a pharmaceutically acceptable nontoxic inert carrier or diluent therefore.

The heteroaroyl-10 deazaaminopterin compound can be used as such, or in the form of an acid addition salt. These salts are formed with one or more free NH_2 groups of the heteroaroyl-10-deazaaminopterin molecule. Typically, the compounds are injected in the form of their sodium salts in aqueous solution. Other salts, e.g., K, Ca, NH_4, and the like, could be used as prepared from the appropriate hydroxide or carbonates.

The acid addition salts are preferable the pharmaceutically acceptable, nontoxic addition salts with suitable acids, such as those with inorganic acids, for example, hydrochloric, hydrobromic, nitric, sulphuric, and phosphoric acids, and with organic acids, such as organic

carboxylic acids, for example, glycolic, maleic, hydroxymaleic, malic, tartaric, citric, salicylic, acetyloxybenzoic, nicotinic, and isonicotinic acid, and organic sulphonic acids, for example, methanesulphonic, ethanesulphonic, 2-hydroxyethanesulphonic, toluene-p-sulphonic, and naphthalene-2-sulphonic acid.

An acid addition salt can be converted into the free compound according to known methods, for example, by treating it with a base, such as with a metal hydroxide or alkoxide, for example, an alkali metal or alkaline earth metal hydroxide, for example, lithium hydroxide, sodium hydroxide, potassium hydroxide or calcium hydroxide; with a metal carbonate, such as an alkali metal or an alkaline earth metal carbonate or hydrogen carbonate, for example, sodium, potassium or calcium carbonate or hydrogen carbonate, with ammonia; or with a hydroxyl ion exchange resin, or with any other suitable reagent.

An acid addition salt may also be converted into another acid addition salt according to known methods, for example, a salt with an inorganic acid may be treated with a metal salt, for example a sodium, barium or silver salt, of an acid in a suitable diluent, in which a resulting inorganic salt is insoluble and is thus removed from the reaction medium. An acid-addition salt may also be converted into another acid addition salt by treatment with an anion exchange preparation.

The glutamic acid COOH groups can also be in salt form, as the ammonium NH_4, alkali metal salts (Na^+, K^+), or the nontoxic alkaline earth metal salts (Ca^{++}) of the glutamate COOH groups.

The heteroaroyl-10-deazaaminopterin compound or salt thereof can be administered to the animal by any available route, including oral and parenteral (intravenous, intraperitoneal, subcutaneous, and intramuscular) administration. The amount administered is sufficient to ameliorate the arthritis or other proliferative disease, and will depend upon the type of arthritis, the species of animal, and the weight of the animal. For example, in human administration, a dosage of heteroaroyl-10-deazaaminopterin compound within the range from about 0.1 mg/kg to about 500 mg/kg per day should be sufficient. Dosages in the higher part of the range, approaching 500 mg/kg, are normally administered in conjunction with leucovorin (dl-r-formyl tetrahydrofolate) to reduce toxicity. In the treatment of lower test animals, a similar dosage range is therapeutic. The upper limit of dosage is that imposed by toxic side effects, and can be determined by trial and error for the animal to be treated, including humans.

To facilitate administration, the heteroaroyl-10-deazaaminopterin compound or salt thereof can be provided in composition form, and preferably in dosage unit form. While the compound can be administered per se, it is normally administered in conjunction with a pharmaceutically acceptable carrier therefor, which dilutes the compound and facilitates handling. The term "pharmaceutically acceptable" means that the carrier (as well as the resulting composition) is sterile and nontoxic.

The carrier or diluent can be solid, semisolid, or liquid, and can serve as a vehicle, excipient, or medium for the heteroaroyl-10-deazaaminopterin compound. Exemplary diluents and carriers include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gun acacia, calcium phosphate, mineral oil, cocoa butter, oil of theobroma, alginates, tragacanth, propyhydroxybenzoate, talc, or magnesium stearate.

For convenience in handling, the heteroaroyl-10-deazaaminopterin compound and carrier or diluent can be enclosed or encapsulated in a capsule, sachet, cachet, gelatin, paper or other container, especially when intended for use in dosage units. The dosage units can for example take the form of tablets, capsules, suppositories, or cachets.

The following Examples 1-7 illustrate various forms of dosage units in which the heteroaroyl-10-deazaaminopterin compounds or salts thereof can be prepared:

Example 1

Tablet Formation	Mg/tablet
Heteroaroyl-10-deazaaminopterin compound	15
Lactose	86
Corn starch (dried)	45.5
Gelatin	2.5
Magnesium stearate	1.0

The heteroaroyl-10-deazaaminopterin compound is powdered and passed through a mesh sieve and well mixed with the lactose and 30 mg of the corn starch, both passed through a sieve.

The mixed powders are massed with a warm gelatin solution, prepared by stirring the gelatin in water and heating to form a 10% w/w solution. The mass is granulated by passing through a sieve, and the moist granules dried at 40°C.

The dried granules are regranulated by passing through a sieve and the balance of the starch and the magnesium stearate is added and thoroughly mixed.

The granules are compressed to produce tablets each weighing 150 mg.

Example 2

Tablet Formation	Mg/tablet
Heteroaroyl-10-deazaaminopterin compound	100
Lactose	39
Corn starch (dried)	80
Gelatin	4.0
Magnesium stearate	2.0

The method of preparation is identical with that of Example 1, except that 60 mg of starch is used in the granulation process and 20 mg during tableting.

Example 3

Capsule formation	Mg/capsule
Heteroaroyl-10-deazaaminopterin compound	250
Lactose	150

The heteroaroyl-10-deazaaminopterin compound and lactose are passed through a sieve and the powders well mixed together before filling into hard gelatin capsules of suitable size, so that each capsule contains 400 mg of mixed powders.

Example 4

Suppositories	Mg/suppositories
Heteroaroyl-10-deazaaminopterin compound	50
Oil of Theobroma	950

The heteroaroyl-10-deazaaminopterin compound is powdered and passed through a sieve and triturated with molten oil of theobroma at 45°C to form a smooth suspension.

The mixture is well stirred and poured into molds, each of nominal 1 g capacity, to product suppositories.

Example 5

Cachets	Mg/cachet
Heteroaroyl-10-deazaaminopterin compound	100
Lactose	400

The heteroaroyl-10-deazaaminopterin compound is passed through a mesh sieve, mixed with lactose previously sieved and fitted into cachets of suitable size so that each contains 500 mg.

Example 6

Intramuscular injection (sterile suspension in aqueous vehicle)	Mg
Heteroaroyl-10-deazaaminopterin compound	10
Sodium citrate	5.7
Sodium carboxymethylcellulose (low viscosity grade)	2.0
Methyl para-hydroxybenzoate	1.5
Propyl para-hydroxylbenzoate	0.2
Water for injection to 1.0 ml	

Example 7

Intraperitoneal intravenous or subcutaneous injection (sterile solution in aqueous carrier system)	Mg
Heteroaroyl-10-deazaaminopterin compound, hydrochloric acid addition salt	15
Sodium citrate	5.7
Sodium carboxymethylcellulose (low viscosity grade)	2.0
Methyl para-hydroxybenzoate	1.5
Propyl para-hydroxylbenzoate	0.2
Water for injection to 1.0 ml	

Example 8

In Vivo Biology of Type II Collagen Arthritis and Methotrexate (MTX) Treatment using Heteroaroyl-10-Deazaaminopterin Compound Nos. 1 to 4 of Table I

The following data illustrate administration to mice of compound Nos. 1 to 4 of Table I, of the invention and methotrexate in the evaluation of anti-inflammatory activity. The data are presented as two separate observations, the visually observed presence of inflammation in the mouse, and the caliper-measured degree of swelling of the rear paws of the mouse.

The efficacy evaluation used a mouse model of inflammatory disease that occurs in response to an antigenic challenge with Type II collagen [J. S. Courtenay, M. J. Dallman, A. D. Dayan, A. Nortin, and B. Mosedale, Nature, 283, 666-668 (1980)].

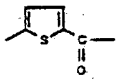
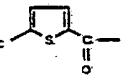
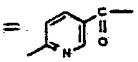
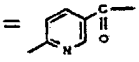
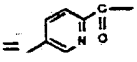
The fundamental aspects of the model allow it to serve as a representative presentation of human disease. The parallels between the known aspects of the mouse model and rheumatoid arthritis include a humoral response in which antibodies are produced to an antigen that is present in the joint tissue and the antigenic challenge is accompanied by cell-mediated aspects of immunity. The resultant inflammation of the joint tissue yields facets of periostitis, synovial lining hyperplasia, degradation of bone and cartilage and pannus and new bone formation.

The basic elements of the model included the immunization of DBA/1 mice with a suspension of fetal bovine Type II collagen (1 mg/ml) prepared in complete Freund's adjuvant. The primary injection was given using 0.1 ml of the collagen emulsion giving a total of 0.1 mg of Type II collagen per mouse. The animals were then given a booster injection of Type II collagen (100 μg in 0.01 M acetic acid) on day 21 by intraperitoneal injection.

The results of the *in vivo* testing of methotrexate showed that using prophylactic regimens in which drug was begun two days prior to administration of antigen (Type II collagen) was more effective than starting drug at day 19, two days prior to the first and only boost with Type II collagen. Typically, in this model the untreated positive control animals have an incidence of arthritis ranging from 90 to 100% of injected animals at day 44.

The effect of methotrexate and test compounds on the extent of inflammation was determined by direct analysis of paw swelling using caliper measurements. The results are presented in Table II, and show a direct correlation between the decrease in the number of animals having disease and a decrease in the extent of inflammation, as determined by paw swelling.

Table II

Compound	Dose mg/kg	No mice affected on day indicated[b] Day 30	Day 37	Day 44	Avg. thickness of rear paws (mm) over days 30-44[c] Treated	Untreated
None		31/43	38/43	41/43		2.29-2.73
1 R = H X = 	18.0	0/8	1/8	2/8	2.14-2.38	
2 R = C_2H_5 X = 	15.0	0/8	1/8	1/8	2.15-2.26	
3 R = H X = 	8.0	3/8	2/8	4/8	2.22-2.33	
4 R = C_2H_5 X = 	2.5	2/8	6/8	6/8	2.18-2.75	
5 R = H X = 						
MTX[a]	9.0	1/22	1/22	6/22	(2.18-2.34)	

a MTX and untreated controls are composites from multiple runs.

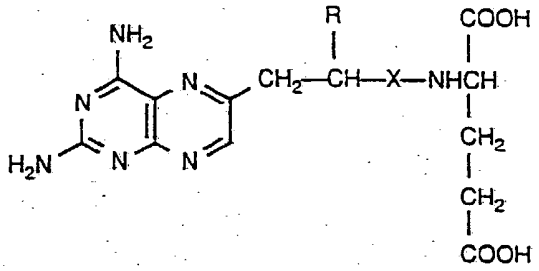
b Visual evidence of inflammation.

c Values in parentheses are 30 day and 44 day measurements vs. equivalent for untreated controls; decrease in inflammation vs. control is most notable at day 44.

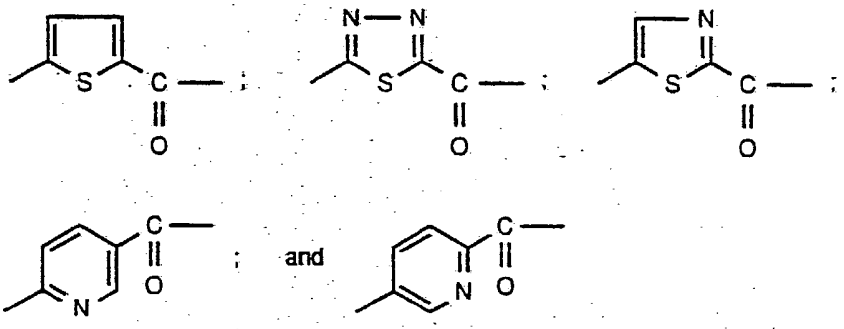
It is apparent from the above results that the number of test mice affected was very considerably decreased by administration of heteroaroyl-10-deazaaminopterin compound. The results show that heteroaroyl-10-deazaaminopterin compound on a similar dosage level to be at least as effective as methotrexate, and since methotrexate is accepted as effective the heteroaroyl-10-deazaaminopterin compound is to be expected to be at least as effective as methotrexate, under similar conditions. The potent anti-arthritic activity of the heteroaroyl-10-deazaaminopterin compounds tested is evident from the results.

WHAT IS CLAIMED IS:

1. Heteroaroyl-10-deazaaminopterin compounds having the formula:



wherein X is one of



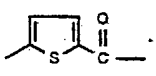
and R is hydrogen or alkyl, alkenyl, or alkynyl having from one to about eight carbon atoms.

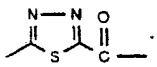
2. The compounds of Claim 1 wherein R is alkenyl .

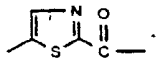
3. The compounds of Claim 1 wherein R is alkynyl.

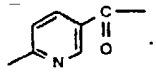
4. The compounds of Claim 1 wherein R is alkyl.

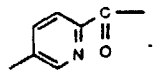
5. The compounds of Claim 4 wherein the alkyl is ethyl.

6. The compounds of Claim 1 wherein X is  .

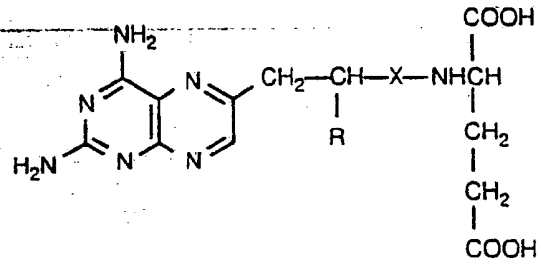
7. The compounds of Claim 1 wherein X is  .

8. The compounds of Claim 1 wherein X is  .

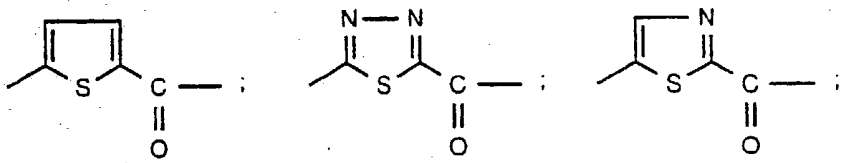
9. The compounds of Claim 1 wherein X is  .

10. The compounds of Claim 1 wherein X is  .

11. A method for treating arthritis and other proliferative diseases which comprises administering to a warm-blooded animal having an inflammation of the joints or other evidence of the diseases, a therapeutic and relatively nontoxic amount of a heteroaroyl-10-deazaaminopterin compound having the formula:



wherein X is one of



and R is hydrogen or alkyl, alkenyl, or alkynyl having from one to about eight carbon atoms.

12. The method of Claim 11 wherein the compound is administered as a pharmaceutically acceptable salt thereof.

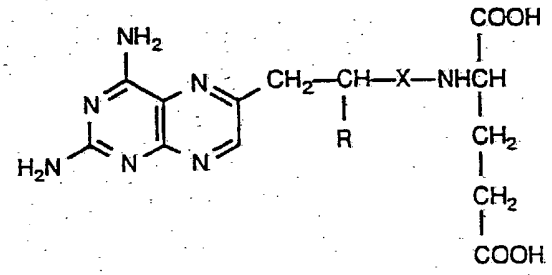
13. The method of Claim 11 wherein the compound is administered in an amount with the range from about 0.1 to about 500 mg per day.

14. The method of Claim 11 wherein the compound is administered with an inert diluent or carrier.

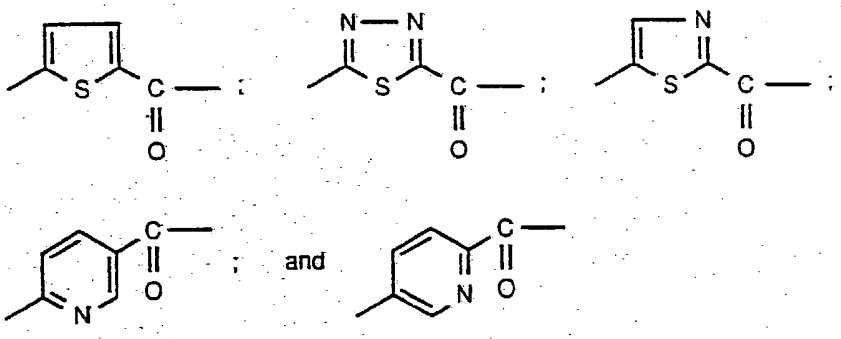
15. The method of Claim 11 wherein the compound is administered orally.

16. The method of Claim 11 wherein the compound is administered parenterally.

17. A pharmaceutical composition in dosage unit form for treating arthritis or other proliferative disease comprising an amount within the range from about 0.1 to about 500 mg per dosage unit therapeutically effective to ameliorate arthritis or other proliferative disease of a heteroaroyl-10-deazaaminopterin compound together with a pharmaceutically acceptable nontoxic carrier or diluent thereof; the heteroaroyl-10-deazaaminopterin compound having the formula:



wherein X is one of



and R is hydrogen or alkyl, alkenyl, or alkynyl having from one to about eight carbon atoms.

18. The pharmaceutical composition of Claim 17 wherein the compound is in the form of a pharmaceutically acceptable acid addition salt.

19. The pharmaceutical composition of Claim 17 or 18 in sterile aqueous, aqueous dispersion, capsule, cachet, or suppository form.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/03963

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07D475/08; A61K31/525

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07D

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
A	WO,A,8 704 161 (SRI INTERNATIONAL) 16 July 1987 *Document*	1-19
A	JOURNAL OF MEDICINAL CHEMISTRY vol. 17, no. 5, May 1974, WASHINGTON pages 552 - 553 JOSEPH I. DEGRAW ET. AL. cited in the application	1-19
A	GB,A,2 058 770 (SRI INTERNATIONAL) 15 April 1981 *Document*	1-19
A	& US,A,4 369 319 (JOSEPH IRVING DE GRAW ET. AL.) cited in the application	1-19

[10] Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
10 AUGUST 1993	17. 08. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	LUYTEN H.W.

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/US93/03963

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 12-16 are directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9303963
SA 73625

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 10/08/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-8704161	16-07-87	US-A-	4746659	24-05-88
		DE-T-	3690639	19-11-87
		EP-A-	0254726	03-02-88
		GB-A,B	2192888	27-01-88
		JP-T-	63502892	27-10-88
GB-A-2058770	15-04-81	US-A-	4393064	12-07-83
		AU-B-	540326	15-11-84
		AU-A-	6243980	26-03-81
		BE-A-	885255	16-01-81
		CA-A-	1139752	18-01-83
		DE-A,C	3034843	11-06-81
		FR-A,B	2464956	20-03-81
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		JP-A-	3034979	14-02-91
		JP-B-	3040029	17-06-91
		US-A-	4369319	18-01-83